PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 09 March 2000 (09.03.00)	
International application No. PCT/DK99/00408	**Applicant's or agent's file reference** 21129 PC 1
International filing date (day/month/year) 16 July 1999 (16.07.99)	**Priority date** (day/month/year) 20 July 1998 (20.07.98)
Applicant HALKIER, Torben et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

03 February 2000 (03.02.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Nestor Santesso
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Form PCT/IB/331 (July 1992)

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PATENT COOPERATION TREATY

PLOUGMANN VINGTOFT & PARTNERS
12 JULI 2000

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
21129 PC 1		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DK99/00408	16/07/1999	20/07/1998
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant M&E BIOTECH A/S et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 11 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03/02/2000	10.07.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Hoesel, H Telephone No. +49 89 2399 8693

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/DK99/00408

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-96 as originally filed

Claims, No.:

1-53 as received on 26/06/2000 with letter of 23/06/2000

Drawings, sheets:

1/7-7/7 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1 - 40
	No:	Claims 41 - 53
Inventive step (IS)	Yes:	Claims 1 - 40
	No:	Claims 41 - 53
Industrial applicability (IA)	Yes:	Claims 1 - 53
	No:	Claims

2. Citations and explanations

see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Reference is made to the following documents:

D1: WO-A-97/27212
D2: WO-A-96/38553
D3: WO-A-98/32880

SECTION I:

Sequence listing pages 1 - 15 as originally filed have been also taken into account as basis of this opinion.

SECTION V:

1. Scope and novelty of claim 1:

   Claim 1 recites the conventional steps of cell-based screening methods for bio-active peptides from peptide libraries but is now limited to the use of a scaffold that has been derived protein which directly modulates the activity of a protease.

   Thereby the method is distinguished with respect to those described in D1 and D2, which documents are considered to represent the closest prior art.
   Thus, the subject-matter of claims 1 and its truly dependent claims (i.e. claims 2 - 40) is novel in the sense of Art. 33(2) PCT.

2. This subject-matter is also deemed to be inventive as required by Art. 33(3) PCT, since neither the choice of the scaffolding structure nor its effects to confer protease resistance to the peptide insert to be screened is disclosed or suggested by the prior art cited in the international search report.

3. Contrary to claim 1, none of claims 41 - 53 is similarly limited.

   It is maintained that the identification of a modulator does not confer any structural or sequence limitation to the modulator peptide and is not part of a method for the preparation of a replication vector, which per se comprises only functional incorporation of an isolated nucleic acid encoding the modulator peptide(see also

Section VIII).

Consequently, claims 41 and higher concern in their present wording the provision by conventional recombinant technology or conventional uses of an unlimited number of peptide modulators.

4. D1 discloses screening for and identification of bioactive modulators that rely on the same or similar methods including the preparation of libraries retroviral expression vectors and cellular libraries obtained by transduction of target cells with the said retroviral library (D1 claims 1 - 22). The method of preparation of the vector or of transformed cells, or the provision of the modulator by recombinant expression cannot be distinguished from those covered by present claims 41 - 43 (Art. 33(2) PCT).

D1 furthermore concerns the use of modulators identified in a first round for identification of their in vivo target molecules or for further rounds of screening (p. 34, line17 - p. 35, lines 25). It thereby anticipates the subject-matter of claims 44 - 53 (Art. 33(2) and (3) PCT.

SECTION VI:

5. D3 published on 30.7.1998 has been filed on 23.01.1998, i.e. prior to the priority date of the present application. Its content will have to be taken into account in the regional phase.

SECTION VIII:

6. Independent claims 41 - 44, 46, 50, 52, 53 contain features that are irrelevant for the category as apparent from the heading of the claim. These features raise thus uncertainty as to the scope and category of the claims, contrary to Art. 6 PCT.

The objection particularly concerns the wording "the method comprising identifying a modulator" in claim 41, steps I, II, and Ia in claim 43 (which steps are irrelevant for enabling a chemical synthesis) or the wording "which has been de novo isolated ..." in claim 50.

Definition of a product, in terms of the process by which it is identified, moreover does not confer any limitation with respect to known bioactive peptides. Consequently, the references included in claims 41 - 44, 46 and 53 raise uncertainty as to the actual scope of protection.

The said claims lack clarity contrary to Art. 56 EPC and do not impart any clear limitation as to the prior art uses of modulators in screening and development of pharmaceutical products.

Should the claims be interpreted to cover the same protection as claim 1, they are superfluous. However, if intended to cover the per se conventional preparation and use of an unlimited number of possible peptide modulators, as it is to be derived from the heading of the said claims, they are not limited with respect to the state of the art (see Section V).

CLAIMS

1. A method for identifying a modulator in the form of a biologically active polypeptide fragment which is capable of detectably modulating, *in vivo*, a phenotypic trait in a cell, the method comprising the steps of

(a) preparing a pool of expression vectors, each vector of said pool containing at least one member from a library of randomly modified nucleotide sequences derived from a parent nucleotide sequence encoding a parent peptide which *in vivo* directly modulates activity of a known protease, wherein the randomly modified nucleotide sequences comprise

- an invariable part encoding a scaffold portion of the parent peptide, said scaffold portion serving to stabilize said polypeptide fragment and being stable towards proteolytic attack and/or being insensitive to a reducing environment, and
- random nucleotides,

(b) transforming a population of substantially identical cells with said vectors of said pool so as to obtain transformed cells,

(c) culturing said transformed cells under conditions facilitating expression of said randomly modified nucleotide sequences,

(d) examining said transformed cells and isolating transformed cell(s) wherein the preselected phenotypic trait is modulated thereby indicating that the expression product of said randomly modified nucleotide sequence is biologically active, and

(e) identifying the modulator by determining said randomly modified nucleotide sequence of said vector present in cell(s) isolated in step (d) and/or determining the amino acid sequence of the expression product encoded by said randomly modified nucleotide sequence.

2. The method according to claim 1, wherein the substantially identical cells are prokaryotic cells.

3. The method according to claim 1, wherein the substantially identical cells are eukaryotic cells.

4. The method according to claim 3, wherein the eukaryotic cells are selected from the group consisting of fungal cells, protozoan cells, animal cells, and plant cells.

5. The method according to claim 4, wherein the animal cells are selected from the group consisting of mammalian cells, arthropod cells such as insect cells, avian cells, and piscine cells.

6. The method according to any of the preceding claims, wherein the transformed cells examined in step (d) predominantly carries one single copy of the vector.

7. The method according to claim 6, wherein transformation step (b) is performed under such conditions that the cells transformed are predominantly or at most transformed with one single vector from said pool, or wherein, prior to carrying out step (d), cells being transformed with more than one vector from said pool are substantially excluded from the further steps.

8. The method according to any one of the preceding claims, wherein the random nucleotides are introduced in part(s) of the parent nucleotide sequence which encode(s) the active site(s) of the parent peptide, or the part(s) which encode(s) structure(s) interfering with the active site(s).

9. The method according to any one of the preceding claims, wherein the invariable part of the nucleotide sequence encodes truncated parts of the scaffold portion of the parent peptide sufficient to maintain stability.

10. The method according to any of the preceding claims, wherein the invariable part of the parent nucleotide sequence encodes a peptide which is free from disulfide bridges.

11. The method according to any one of claims 1-9, wherein the invariable part of the parent nucleotide sequence encodes a peptide having disulfide bridges.

12. The method according to any one of the preceding claims, wherein the random nucleotides are introduced in the form of an insertion or a substitution into the parent nucleotide sequence, optionally in combination with deletion(s) in the parent nucleotide sequence.

13. The method according to claim 12, wherein the number of random nucleotides which are introduced is in the range from 3 to about 100.

14. The method according to any one of the preceding claims, wherein the random nucleotides are nucleotide sequences and/or are single random nucleotides introduced at specific sites in the parent nucleotide sequence.

15. The method according to any one of the preceding claims, wherein the random nucleotides are selected from the group consisting of

- synthetic, completely random deoxyribonucleotides;
- synthetic random DNA sequences, wherein limitation on randomization of some nucleotides is introduced so as to limit the number of available sequences and/or to avoid undesired stop codons and/or to facilitate introduction of post-translational modifications of expressed peptide(s);
- synthetic random DNA sequences as in (1) or (2) coupled to a sequence encoding a purification tag; and
- CDR encoding nucleotide sequences isolated from a library of immune-competent cells raised against an antigen.

16. The method according to claim 15, wherein the CDR encoding nucleotide sequences encode CDR-3 peptide sequences.

17. The method according to any one of claims 14-16, wherein the random nucleotides are prepared by random codon synthesis where defined DNA codons are synthesized in a random order.

18. The method according to claim 17, wherein the relative amount of synthesized codons ensure that all encoded amino acids will be present with substantially the same frequency in the total of encoded polypeptide fragments.

19. The method according to any one of the preceding claims, wherein the random nucleotides are introduced into the expression vector by the principle of site directed PCR-mediated mutagenesis.

20. The method according to any one of the preceding claims, wherein the parent peptide is an inhibitor of activity of the known protease.

21. A method according to claim 20, wherein the inhibitor is selected from the group consisting of a BPTI/Kunitz family protease inhibitor, a serpin family protease inhibitor, a Kazal family protease inhibitor, a soybean trypsin inhibitor (Kunitz) family protease inhibitor, a potato inhibitor I family member, a Bowman-Birk family protease inhibitor, a squash inhibitor family member, a wap-type 'Four-disulfide Core' proteinase inhibitor, a hirudin family protease inhibitor, a factor Xa inhibitor, an Ascaris trypsin inhibitor family member, a cystatin family protease inhibitor, a calpain family cysteine protease inhibitor, a tissue inhibitor of metalloproteinases family member, a carboxypeptidase A inhibitor, a metallocarboxypeptidase inhibitor, and an angiotensin-converting enzyme inhibitor.

22. The method according to claim 21, wherein the parent peptide is a potato inhibitor family I member.

23. The method according to claim 22, wherein the parent peptide is chymotrypsin inhibitor 2A (CI-2A).

24. The method according to any of the preceding claims wherein the substantially identical cells are mammalian cells and the vector is selected from the group consisting of a retroviral vector, a vaccinia virus vector, an adenoviral vector, an adeno associated virus (AAV) vector, a herpes simplex virus (HSV) vector, an alpha virus vector, and a semliki forest virus vector.

25. The method according to claim 24, wherein the vector is retroviral.

26. The method according to claim 25, wherein the retroviral vector is derived from retrovirus selected from the group consisting of Avian Leukosis-Sarcoma Virus (ALSV), Mammalian type C, Mammalian type B, and Lentivirus, and optionally modified with heterologous cis-acting elements.

27. The method according to claim 25 or 26, wherein the retroviral vector has non-identical ends.

28. The method according to claim 27, wherein the non-identical ends contain non-identical promoters.

29. The method according to any one of claims 25-28, wherein the retroviral vector contains a heterologous promoter replacing the viral promoter in the 5'-LTR, such as a CMV promoter, an RSV promoter, an SV-40 promoter, a TK promoter, an MT promoter, or an inducible system such as Tet or Ecdysone.

30. The method according to any one of claims 25-29, wherein step (a) is carried out by

1) transfecting suitable packaging cells with vectors which comprise the randomly modified nucleotide sequences and which are integratable in virions produced by said packaging cells,
2) culturing said transfected packaging cells in a culture medium under conditions which facilitate production by the packaging cells of virions containing the randomly modified nucleotide sequences,

3) recovering and optionally concentrating said virions, and
4) transducing said substantially identical cells with the virions.

31. The method according to claim 30, wherein the packaging cells are selected from the group consisting of PE501, Bosc23, Ψ2, GP+E86, PhoenixEco, PA317, GP+AM12, DA(ampho), Bing, FLYA13, ProPak, CRIP, ΨAM, Phoenix-Ampho, PG13, H9 (293GPG), and EcoPack.

32. The method according to any one of claims 25-31, wherein the virions are pseudotyped retrovirus produced by an ecotropic packaging cell line so as to confer broad tropism to the virions produced thereby, or wherein an ecotropic receptor has been introduced into the substantially identical cells so as to allow transduction with ecotropic virions.

33. The method according to claim 32, wherein the ecotropic receptor has been introduced in the substantially identical cells by means of transduction.

34. The method according to any of the preceding claims wherein the randomly modified nucleotide sequences are coupled to a nucleotide sequence encoding at least one fusion partner.

35. The method according to claim 34, wherein the fusion partner serves to facilitate expression and/or purification/isolation and/or further stabilization of the expression product.

36. The method according to claim 35, wherein the fusion partner includes a purification tag such as His_6 tag, myc tag,

BSP biotinylation target sequence, of BirA, flu tag, lacZ, and GST.

37. The method according to claim 34 or 35, wherein the fusion partner is a sorting signal or a targeting sequence.

38. The method according to claim 37, wherein the sorting signal is a signal patch or a signal peptide.

39. The method according to claim 37 or 38, wherein the sorting signal effects export of the expressed peptide out of the cell or into the cell membrane, or, when the substantially identical cells are eukaryotic, into endoplasmic reticulum, into Golgi apparatus, into lysosomes, into secretory vesicles, into mitochondria, into peroxisomes, or into the nucleus.

40. The method according to any one of the preceding claims, which further comprises the step of resolving the 3-dimensional structure of the identified modulator.

41. A method for the preparation of a replicable expression vector, the method comprising the steps of identifying a modulator by the method according to any one of the preceding claims, and subsequently

i) isolating or synthesizing a nucleic acid sequence which encodes the modulator, and

ii) engineering a replicable expression vector comprising an operon which comprises, in the 5'-3' direction and in operable linkage, 1) a promoter for driving expression of the nucleic acid sequence, 2) optionally a nucleotide sequence encoding a leader peptide, 3) the

nucleic acid sequence, and 4) optionally a termination signal.

42. A method for the preparation of a transformed cell carrying a nucleic acid sequence encoding a modulator as defined in any one of claims 1-40, the method comprising transforming a suitable host cell with an expression vector prepared according to claim 41.

43. A method for providing a modulator as defined in any one of claims 1-40, the method comprising

I) growing a transformed cell prepared according to the method of claim 42 in a culture medium under conditions which facilitates expression by the cell of the randomly modified nucleotide sequence, and

II) subsequently harvesting the expression product from the cell and/or the culture medium, or

Ia) identifying the modulator according to the method of any one of claims 1-40, and

Ib) subsequently synthesizing the modulator by means of chemical synthesis on the basis of the sequence determined in step (e).

44. A method for isolating and/or identifying a target biomolecule, the method comprising providing a modulator according to the method of claim 43 and subsequently using the modulator as an affinity ligand in an affinity purification step so as to isolate the target biomolecule from a suitable sample.

45. The method according to claim 44, wherein the affinity purification step is an affinity chromatographic step, an

affinity mass spectrometry step, or a co-immunoprecipitation step.

46. A method for isolating and/or identifying a target biomolecule, the method comprising providing a peptide modulator according to the method of claim 42 and subsequently using the modulator as a probe against a cDNA library derived from the substantially identical cells or using the modulator as bait in a two- or three-hybrid system.

47. The method according to any of claims44-46, wherein the target biomolecule is a peptide or a nucleic acid.

48. The method according to claim 47 further comprising the step of determining the amino acid sequence of the peptide or determining the nucleotide sequence of the nucleic acid.

49. The method according to any of claims 44-48, further comprising the step of resolving the 3-dimensional structure of the target biomolecule.

50. A method for selecting a chemical compound as a putative drug candidate in drug development, the method comprising the steps of

- assaying a library of chemical compounds for interaction with a target biomolecule which has been de novo isolated according to the method of any one of claims 44-49, and
- selecting compounds which interact significantly with the target biomolecule.

51. The method according to claim 50, wherein the library of chemical compounds has been provided by chemical synthesis

upon initial identification of the members of the library by structure-based or non-structure based computer drug-modelling.

52. A method for the preparation of a medicinal product, the method comprising the steps of

A) selecting a chemical compound by the method according to claim 50 or 51,

B) performing pre-clinical tests with the chemical compound in order to assess the suitability thereof as a medicinal product,

C) entering, if the chemical compound is deemed suitable in step (B), clinical trials using the chemical compound in order to obtain market authorization for a medicinal product including the lead compound as a pharmaceutically active substance, and

D) upon grant of a market authorization, admixing the chemical compound with a pharmaceutically acceptable carrier, excipient or diluent and marketing the thus obtained medicinal product.

53. A method for developing a medicinal product, the method comprising that a modulator identified according to the method of any one of claims 1-40 serves as a lead compound in the drug development phase or wherein a target biomolecule isolated/identified according to any one of claims 44-49 serves as an interaction probe for the identification of putative drug candidates in the drug discovery phase.

CLAIMS

1. A method for identifying an *in vivo* active modulator of activity of a target enzyme, the method comprising the steps of

(a) preparing a pool of expression vectors, each vector of said pool containing at least one member from a library of randomly modified nucleotide sequences derived from a parent nucleotide sequence encoding a parent peptide or parent ribonucleic acid which modulates the target enzyme activity,

(b) transforming a population of substantially identical cells with said vectors of said pool so as to obtain transformed cells, said substantially identical cells being ones which harbour the target enzyme,

(c) culturing said transformed cells under conditions facilitating expression of said randomly modified nucleotide sequences,

(d) examining said transformed cells and isolating transformed cell(s) wherein the activity of the target enzyme is modulated, and

(e) identifying the modulator by determining said randomly modified nucleotide sequence of said vector present in cell(s) isolated in step (d) and/or determining the amino acid sequence or the ribonucleic acid sequence of the expression product encoded by said randomly modified nucleotide sequence.

2. The method according to claim 1, wherein the randomly modified nucleotide sequences consist of 1) an invariable part of the parent nucleotide sequence, and 2) random nucleotides.

3. The method according to claim 2, wherein the invariable part of the parent nucleotide sequence encodes a scaffold portion of the parent peptide or of the parent ribonucleic acid which serves to stabilize said polypeptide fragment or ribonucleic acid fragment.

4. A method for identifying a modulator in the form of a biologically active polypeptide fragment or ribonucleic acid fragment which is capable of detectably modulating, *in vivo*, a phenotypic trait in a cell, the method comprising the steps of

(a) preparing a pool of expression vectors, each vector of said pool containing at least one member from a library of randomly modified nucleotide sequences derived from a parent nucleotide sequence encoding a parent peptide or parent ribonucleic acid which *in vivo* modulates activity of a known enzyme, wherein the randomly modified nucleotide sequences comprise

- an invariable part encoding a scaffold portion of the parent peptide or of the parent ribonucleic acid, said scaffold portion serving to stabilize said polypeptide fragment or ribonucleic acid fragment, and
- random nucleotides,

(b) transforming a population of substantially identical cells with said vectors of said pool so as to obtain transformed cells,

(c) culturing said transformed cells under conditions facilitating expression of said randomly modified nucleotide sequences,

(d) examining said transformed cells and isolating transformed cell(s) wherein the preselected phenotypic trait is modulated thereby indicating that the expression product of said randomly modified nucleotide sequence is biologically active, and

(e) identifying the modulator by determining said randomly modified nucleotide sequence of said vector present in cell(s) isolated in step (d) and/or determining the amino acid sequence or the ribonucleic acid sequence of the expression product encoded by said randomly modified nucleotide sequence.

5. The method according to any of the preceding claims wherein the substantially identical cells are prokaryotic cells.

6. The method according to any one of claims 1-4, wherein the substantially identical cells are eukaryotic cells.

7. The method according to claim 6, wherein the eukaryotic cells are selected from the group consisting of fungal cells, protozoan cells, animal cells, and plant cells.

8. The method according to claim 7, wherein the animal cells are selected from the group consisting of mammalian cells, arthropod cells such as insect cells, avian cells, and piscine cells.

9. The method according to any of the preceding claims, wherein the transformed cells examined in step (d) predominantly carries one single copy of the vector.

10. The method according to claim 9, wherein transformation step (b) is performed under such conditions that the cells transformed are predominantly or at most transformed with one single vector from said pool, or wherein, prior to carrying out step (d), cells being transformed with more than one vector from said pool are substantially excluded from the further steps.

11. The method according to any of the preceding claims, wherein the modulator is a peptide.

12. The method according to any of claims 1-10, wherein the modulator is a nucleic acid fragment such as an RNA fragment.

13. The method according to any of the preceding claims, wherein the modulator is stable towards proteolytic attack and/or is insensitive to a reducing environment.

14. The method according to any one of claims 2-13, wherein the random nucleotides are introduced in part(s) of the parent nucleotide sequence which encode(s) the active site(s) of the parent peptide or parent ribonucleic acid, or the part(s) which encode(s) structure(s) interfering with the active site(s).

15. The method according to any one of claims 2-14, wherein the invariable part of the nucleotide sequence encodes truncated parts of the parent peptide or parent ribonucleic acid sufficient to maintain stability.

16. The method according to any of claims 2-15, wherein the invariable part of the parent nucleotide sequence encodes a peptide which is free from disulfide bridges.

17. The method according to any one of claims 2-15, wherein the invariable part of the parent nucleotide sequence encodes a peptide having disulfide bridges.

18. The method according to any one of claims 2-17, wherein the random nucleotides are introduced in the form of an insertion or a substitution into the parent nucleotide sequence, optionally in combination with deletion(s) in the parent nucleotide sequence.

19. The method according to claim 18, wherein the number of random nucleotides which are introduced is in the range from 3 to about 100.

20. The method according to any one of claims 2-19, wherein the random nucleotides are nucleotide sequences and/or are single random nucleotides introduced at specific sites in the parent nucleotide sequence.

21. The method according to any one of claims 2-20, wherein the random nucleotides are selected from the group consisting of

- synthetic, completely random deoxyribonucleotides;

- synthetic random DNA sequences, wherein limitation on randomization of some nucleotides is introduced so as to limit the number of available sequences and/or to avoid undesired stop codons and/or to facilitate introduction of post-translational modifications of expressed peptide(s);

- synthetic random DNA sequences as in (1) or (2) coupled to a sequence encoding a purification tag; and

- CDR encoding nucleotide sequences isolated from a library of immune-competent cells raised against an antigen.

22. The method according to claim 21, wherein the CDR encoding nucleotide sequences encode CDR-3 peptide sequences.

23. The method according to any one of claims 20-22, wherein the random nucleotides are prepared by random codon synthesis where defined DNA codons are synthesized in a random order.

24. The method according to claim 23, wherein the relative amount of synthesized codons ensure that all encoded amino acids will be present with substantially the same frequency in the total of encoded polypeptide fragments.

25. The method according to any one of claims 2-24, wherein the random nucleotides are introduced into the expression vector by the principle of site directed PCR-mediated mutagenesis.

26. The method according to any one of the preceding claims, wherein the modulator *in vivo* reduces or increases K_M of the target enzyme for at least one substrate.

27. The method according to any of the preceding claims, wherein the modulator *in vivo* reduces or increases V_{max} of the target enzyme for at least one substrate.

28. The method according to any one of the preceding claims, wherein the parent peptide or parent ribonucleic acid is an inhibitor of activity of the target enzyme.

29. A method according to claim 28, wherein the inhibitor is selected from the group consisting of

a BPTI/Kunitz family protease inhibitor, a serpin family protease inhibitor, a Kazal family protease inhibitor, a soybean trypsin inhibitor (Kunitz) family protease inhibitor, a potato inhibitor I family member, a Bowman-Birk family protease inhibitor, a squash inhibitor family member, a wap-type 'Four-disulfide Core' proteinase inhibitor, a hirudin family protease inhibitor, a factor Xa inhibitor, an Ascaris trypsin inhibitor family member, a cystatin family protease inhibitor, a calpain family cysteine protease inhibitor, a tissue inhibitor of metalloproteinases family member, a carboxypeptidase A inhibitor, a metallocarboxypeptidase inhibitor, an angiotensin-converting enzyme inhibitor, a cereal alpha-amylase/trypsin inhibitor family member, an alpha-amylase/trypsin inhibitor homologous to thaumatin, an alpha-amylase/subtilisin inhibitor family member, an inhibitors of insect alpha-amylases, an inhibitor of mammalian alpha-amylases derived from Streptomyces species, a trehalase inhibitor, a polygalacturonase inhibitor, a fucosyltransferase inhibitor, a protein kinase C inhibitor, an cAMP-dependent protein kinase inhibitor, a cyclic nucleotide phosphodiesterase inhibitor, a protein phosphatase inhibitor, a TCD/MRS6 family GDP dissociation inhibitor, an ATPase inhibitor, a phospholipase A2 inhibitory protein, a ribonuclease inhibitor, an RNA polymerase inhibitor, a DNA-entry nuclease inhibitor, and a beta-lactamase inhibitor.

30. The method according to any of the preceding claims wherein the substantially identical cells are mammalian cells and the vector is selected from the group consisting of a retroviral vector, a vaccinia virus vector, an adenoviral vector, an adeno associated virus (AAV) vector, a herpes simplex virus (HSV) vector, an alpha virus vector, and a semliki forest virus vector.

31. The method according to claim 30, wherein the vector is retroviral.

32. The method according to claim 31, wherein the retroviral vector is derived from retrovirus selected from the group consisting of Avian Leukosis-Sarcoma Virus (ALSV), Mammalian type C, Mammalian type B, and Lentivirus, and optionally modified with heterologous cis-acting elements.

33. The method according to claim 31 or 32, wherein the retroviral vector has non-identical ends.

34. The method according to claim 33, wherein the non-identical ends contain non-identical promoters.

35. The method according to any one of claims 31-34, wherein the retroviral vector contains a heterologous promoter replacing the viral promoter in the 5'-LTR, such as a CMV promoter, an RSV promoter, an SV-40 promoter, a TK promoter, an MT promoter, or an inducible system such as Tet or Ecdysone.

36. The method according to any one of claims 31-35, wherein step (a) is carried out by

1) transfecting suitable packaging cells with vectors which comprise the randomly modified nucleotide sequences and which are integratable in virions produced by said packaging cells,

2) culturing said transfected packaging cells in a culture medium under conditions which facilitate production by the packaging cells of virions containing the randomly modified nucleotide sequences,

3) recovering and optionally concentrating said virions, and

4) transducing said substantially identical cells with the virions.

37. The method according to claim 36, wherein the packaging cells are selected from the group consisting of PE501, Bosc23, Ψ2, GP+E86, PhoenixEco, PA317, GP+AM12, DA(ampho), Bing, FLYA13, ProPak, CRIP, ΨAM, Phoenix-Ampho, PG13, H9 (293GPG), and EcoPack.

38. The method according to any one of claims 31-37, wherein the virions are pseudotyped retrovirus produced by an ecotropic packaging cell line so as to confer broad tropism to the virions produced thereby, or wherein an ecotropic receptor has been introduced into the substantially identical cells so as to allow transduction with ecotropic virions.

39. The method according to claim 38, wherein the ecotropic receptor has been introduced in the substantially identical cells by means of transduction.

40. The method according to any of the preceding claims wherein the randomly modified nucleotide sequences are coupled to a nucleotide sequence encoding at least one fusion partner.

41. The method according to claim 40, wherein the fusion partner serves to facilitate expression and/or purification/isolation and/or further stabilization of the expression product.

42. The method according to claim 41, wherein the fusion partner includes a purification tag such as His6 tag, myc tag, BSP biotinylation target sequence, of BirA, flu tag, lacZ, and GST.

43. The method according to claim 40 or 41, wherein the fusion partner is a sorting signal or a targeting sequence.

44. The method according to claim 43, wherein the sorting signal is a signal patch or a signal peptide.

45. The method according to claim 43 or 44, wherein the sorting signal effects export of the expressed peptide out of the cell or into the cell membrane, or, when the substantially identical cells are eukaryotic, into endoplasmic reticulum, into Golgi apparatus, into lysosomes, into secretory vesicles, into mitochondria, into peroxisomes, or into the nucleus.

46. The method according to any one of the preceding claims, which further comprises the step of resolving the 3-dimensional structure of the identified modulator.

47. A method for the preparation of a replicable expression vector, the method comprising the steps of identifying a modulator by the method according to any one of claims 1-46, and subsequently

i) isolating or synthesizing a nucleic acid sequence which encodes the modulator, and

ii) engineering a replicable expression vector comprising an operon which comprises, in the 5'-3' direction and in operable linkage, 1) a promoter for driving expression of the nucleic acid sequence, 2) optionally a nucleotide sequence encoding a leader peptide, 3) the nucleic acid sequence, and 4) optionally a termination signal.

48. A method for the preparation of a transformed cell carrying a nucleic acid sequence encoding a modulator as defined in any one of claims 1-46, the method comprising transforming a

suitable host cell with an expression vector prepared according to claim 47.

49. A method for providing a modulator as defined in any one of claims 1-46, the method comprising

I) growing a transformed cell prepared according to the method of claim 48 in a culture medium under conditions which facilitates expression by the cell of the randomly modified nucleotide sequence, and

II) subsequently harvesting the expression product from the cell and/or the culture medium, or

Ia) identifying the modulator according to the method of any one of claims 1-46, and

Ib) subsequently synthesizing the modulator by means of chemical synthesis on the basis of the sequence determined in step (e).

50. A method for isolating and/or identifying a target biomolecule, the method comprising providing a modulator according to the method of claim 49 and subsequently using the modulator as an affinity ligand in an affinity purification step so as to isolate the target biomolecule from a suitable sample.

51. The method according to claim 50, wherein the affinity purification step is an affinity chromatographic step, an affinity mass spectrometry step, or a co-immunoprecipitation step.

52. A method for isolating and/or identifying a target biomolecule, the method comprising providing a peptide modulator according to the method of claim 48 and subsequently using the modulator as a probe against a cDNA library derived from

the substantially identical cells or using the modulator as bait in a two- or three-hybrid system.

53. The method according to any of claims 50-52, wherein the target biomolecule is a peptide or a nucleic acid.

54. The method according to claim 53 further comprising the step of determining the amino acid sequence of the peptide or determining the nucleotide sequence of the nucleic acid.

55. The method according to any of claims 50-54, further comprising the step of resolving the 3-dimensional structure of the target biomolecule.

56. A method for selecting a chemical compound as a putative drug candidate in drug development, the method comprising the steps of

- assaying a library of chemical compounds for interaction with a target biomolecule which has been de novo isolated according to the method of any one of claims 50-55, and
- selecting compounds which interact significantly with the target biomolecule.

57. The method according to claim 56, wherein the library of chemical compounds has been provided by chemical synthesis upon initial identification of the members of the library by structure-based or non-structure based computer drug-modelling.

58. A method for the preparation of a medicinal product, the method comprising the steps of

A) selecting a chemical compound by the method according to claim 56 or 57,

B) performing pre-clinical tests with the chemical compound in order to assess the suitability thereof as a medicinal product,

C) entering, if the chemical compound is deemed suitable in step (B), clinical trials using the chemical compound in order to obtain market authorization for a medicinal product including the lead compound as a pharmaceutically active substance, and

D) upon grant of a market authorization, admixing the chemical compound with a pharmaceutically acceptable carrier, excipient or diluent and marketing the thus obtained medicinal product.

59. A method for developing a medicinal product, the method comprising that a modulator identified according to the method of any one of claims 1-46 serves as a lead compound in the drug development phase or wherein a target biomolecule isolated/identified according to any one of claims 50-55 serves as an interaction probe for the identification of putative drug candidates in the drug discovery phase.

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0	For receiving Office use only	
0-1	International Application No.	
0-2	International Filing Date	
0-3	Name of receiving Office and "PCT International Application"	
0-4	Form - PCT/RO/101 PCT Request	
0-4-1	Prepared using	PCT-EASY Version 2.84 (updated 01.06.1999)
0-5	Petition The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	Receiving Office (specified by the applicant)	Danish Patent Office (RO/DK)
0-7	Applicant's or agent's file reference	21129 PC 1
I	Title of invention	NOVEL METHODS FOR THE IDENTIFICATION OF LIGAND AND TARGET BIOMOLECULES
II	Applicant	
II-1	This person is:	applicant only
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III-2	**Applicant and/or inventor**	
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IV-1	**Agent or common representative; or address for correspondence** The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	agent
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V	**Designation of States**	
V-1	Regional Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AP: GH GM KE LS MW SD SZ UG ZW and any other State which is a Contracting State of the Harare Protocol and of the PCT EA: AM AZ BY KG KZ MD RU TJ TM and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT EP: AT BE CH&LI CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE and any other State which is a Contracting State of the European Patent Convention and of the PCT OA: BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG and any other State which is a member State of OAPI and a Contracting State of the PCT

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V-2	National Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AE AL AM AT (patent and utility model) AU AZ BA BB BG BR BY CA CH&LI CN CU CZ (patent and utility model) DE (patent and utility model) DK (patent and utility model) EE (patent and utility model) ES FI (patent and utility model) GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK (patent and utility model) SL TJ TM TR TT UA UG US UZ VN YU ZA ZW	
V-5	**Precautionary Designation Statement** In addition to the designations made under items V-1, V-2 and V-3, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) of the State(s) indicated under item V-6 below. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit.		
V-6	**Exclusion(s) from precautionary designations**	NONE	
VI-1	**Priority claim of earlier national application**		
VI-1-1	Filing date	20 July 1998 (20.07.1998)	
VI-1-2	Number	PA 1998 00956	
VI-1-3	Country	DK	
VI-2	**Priority claim of earlier national application**		
VI-2-1	Filing date	29 July 1998 (29.07.1998)	
VI-2-2	Number	60/094,868	
VI-2-3	Country	US	
VI-3	**Priority document request** The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s):	VI-1	
VII-1	**International Searching Authority Chosen**	European Patent Office (EPO) (ISA/EP)	
VIII	**Check list**	number of sheets	electronic file(s) attached
VIII-1	Request	4	-
VIII-2	Description (excluding sequence listing part)	96	-
VIII-3	Claims	13	-
VIII-4	Abstract	1	abstract.txt
VIII-5	Drawings	7	-
VIII-6	Sequence listing part of description	15	-
VIII-7	TOTAL	136	

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	Accompanying items	paper document(s) attached	electronic file(s) attached
VIII-8	Fee calculation sheet	✓	–
VIII-9	Separate signed power of attorney	✓	–
VIII-15	Nucleotide and/or amino acid sequence listing in computer readable form		separate diskette
VIII-16	PCT-EASY diskette	–	diskette
VIII-18	Figure of the drawings which should accompany the abstract	1	
VIII-19	Language of filing of the international application	English	
IX-1	Signature of applicant or agent		
IX-1-1	Name (LAST, First)	KOEFOED, Peter	

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10-1	Date of actual receipt of the purported international application	
10-2	Drawings:	
10-2-1	Received	
10-2-2	Not received	
10-3	Corrected date of actual receipt due to later but timely received papers or drawings completing the purported International application	
10-4	Date of timely receipt of the required corrections under PCT Article 11(2)	
10-5	International Searching Authority	ISA/EP
10-6	Transmittal of search copy delayed until search fee is paid	

FOR INTERNATIONAL BUREAU USE ONLY

11-1	Date of receipt of the record copy by the International Bureau	